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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| Serial No. | : 09/836,705 |
| Applicants | : Yuki ABE et al. |
| Filed | : April 17, 2001 |
| Art Unit | : 1652 |
| Examiner | : Kathleen M. Kerr |
| Docket No. | : 01149/HG |
| Customer No. | : 01933 |
| Confirmation No. | : 7090 |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 CFR 1.132**

S I R :

I, Yuki ABE, declare as follows:

1. I am a coinventor of the above-identified patent application.

2. I graduated from the University of Tokyo, Japan in 199 6, and I received a Master's degree in the field of agriculture.

3. I have worked for Sankyo Company, Limited of Tokyo, Japan, since 1996, and I presently a researcher.

4. The following information was generated by experiments that were carried out under my supervision.

Two panels are attached hereto. The two panels show the results of genomic southern blot hybridizations with the mlcR and the mlcE cDNA as probes, respectively. The hybridizations were performed according to Example 5 on pages 42 to 44 of the specification of application Serial No. 09/836,705, the entire contents of which are hereby incorporated by reference herein.

The genomic DNA of *Penicillium citrinum* SANK 13380, *Penicillium chrysogenum*, *Penicillium* notatum, *Penicillium italicum* and *Eupenicillium sinaicum* were digested separately with a restriction enzyme BamH 1 at 37°C for 2 hours and then loaded on lanes 1 to 5 of each gel, respectively.

Only one band was specifically detected in each lane of the left panel, and two bands were specifically detected in each lane of the right panel.

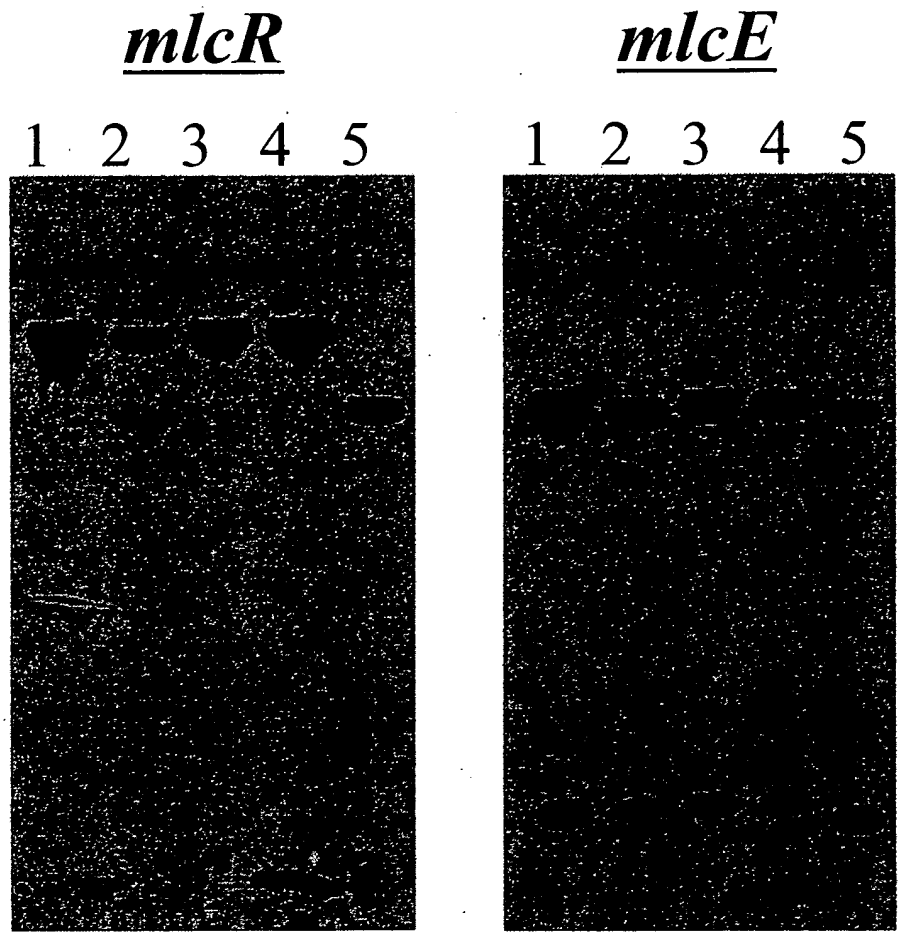
All of the *Penicillium* species *chrysogenum*, *notatum* and *italicum* and the *Eupenicillium* species *sinaicum* were confirmed to produce ML-236B and can be distinguished from *Penicilllium citrinum* on the basis of the morphological and taxonomical analysis.

These results demonstrate that there are genes corresponding to mlcR and mlcE in the genome of ML-236B producing microorganisms and said genes contribute to the production of ML-236B in said microorganisms, and further that ML-236B-producing microorganisms other than *Penicillium citrinum* can be used as a host cell in the claimed methods.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both, under Section 1001, of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: October 6, 2003 By: Yuki Abe
Yuki ABE




1. *Penicillium citirinum*
2. *Penicillium chrysogenum*
3. *Penicilllium notatum*
4. *Penicillium italicum*
5. *Eupenicillium sinaicum*

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AN INTERNATIONAL JOURNAL DESIGNED TO EXPEDITE PUBLICATION OF RESEARCH ON TAXONOMY & NOMENCLATURE OF FUNGI & LICHENS

# A NEW EUPENICILLIUM SPECIES WITH RETICULATELY ORNAMENTED ASCOSPORES

SHUN-ICHI UDAGAWA AND SEIICHI UEDA

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# A NEW EUPENICILLIUM SPECIES WITH RETICULATELY ORNAMENTED ASCOSPORES

Shun-ichi UDAGAWA

*National Institute of Hygienic Sciences, Kamiyoga 1-chome, Setagaya-ku, Tokyo 158, Japan*

AND

Seiichi UEDA

*Nagasaki Prefectural Institute of Public Health and Environmental Sciences, Nameshi 1-chome, Nagasaki 852, Japan*

Members of *Eupenicillium*, one of several groups of ascomycetes having *Penicillium* anamorphs, have been reported to be of wide-spread occurrence throughout the world (Raper and Thom, 1949; Scott, 1968; Pitt, 1979). As with *Talaromyces* species, field soils apparently provide *Eupenicillium* spp. with their most common habitats. They are also frequently encountered in marine environments. Ueda (1980, and a paper presented at the Annual Meeting of the Mycological Society of Japan, held at Niigata, May 28th, 1981) reported a number of *Eupenicillium* species from marine sludges in Nagasaki Bay, southern Japan, and suggested that their presence there might be attributed to sedimentation from surrounding terrestrial habitats. *Eupenicillium brefeldianum* (B.O. Dodge) Stolk & Scott and *E. javanicum* (van Beyma) Stolk & Scott are the most commonly encountered species from marine and estuarine environments.

A member of the genus with reticulately ornamented ascospores was isolated during a continuing study of ascomycetous microfungi in marine sludges. It proved to be sufficiently different from all described taxa of the genus to warrant its description as a new species.

*Eupenicillium sinaicum* Udagawa & Ueda, sp.nov. (Figs. 1-4, 6)

Stat. anam. *Penicillium sinaicum* Udagawa & Ueda, st. nov.

Coloniae in agaro Czapekii satis crescentes, sulcatae vel plus minusve rugosae, e coacto crasso mycelio compositae, floccosae, albae vel roseo-bulbalinae vel roseo-vinosae, numerosis obscuris cleistotheciis et sparsis conidialibus fructificationibus formantes; reversum roseo-bulbalinum vel incarnatum. Coloniae in agaro maltoso effusae, tenues, floccosae vel veltinae, deinde granulosae, croceae; cleistothecia abunde producentia et conidia sparsa; reversum melleum vel isabellinum. Coloniae in agaro farina avenae mixto effusae, tenuiores, granulosae, salmoneae; cleistothecia abunde producentia; reversum incolor vel aliquantum ferrugineum.

Cleistothecia superficialia, dispersa vel confluentia, dilute flavo-brunnea, subglobosa vel elongata, 150-280 µm diam, glabra vel hyphis pigmentiferis laxe intricatis circumdata; peridium primo sclerotioideum, deinde tenue et membranaceum. Asci catenati in ascogonicis hyphis, octospori, subglobosi vel ovati, 9-11.5 × 8-10 µm, postremo evanescentes. Ascosporae hyalinae vel dilute flavae, late lenticulares, 3.0-4.0 × 2.5-3.0 µm, cum subtili aequatoriali sulco praeditae sed sine distincte cristis, reticulatis convexis ornatae (sub 'SEM').

Penicilli monoverticillati vel plerumque biverticillati et asymmetrici. Conidiophora hyalina, levia, septata, variabilia, e substrato erecto 350-450(-900) × 2.5-3.0 (-3.5) µm, vel e hyphis aeriis oriunda usque 80 µm longa. Rami interdum praediti, 25-35 µm longi. Metulae 2-4 per verticillum, 12.5-15(-20) × 2.5-4.0 µm, hyalinae, leves. Phialides 3-4 per verticillum, 9-12.5 × 2.0-2.5 µm. Conidia hyalina, globosa vel subglobosa, 2.0-3.0 µm diam, levia, catenata. Chlamydosporae abunde producentes, vulgo intercalares.

Holotypus No. 2894, NHL, isolatus e limo mari in 'Port Said', Aegypto, 9.x.1978, leg. H. Komatsu. In herb. NHL.

Etymology: lat. *sinaicum*, referring to the Sinai peninsula, Egypt, the type locality.

Colonies on Czapek agar growing moderately, attaining a diameter of 4.0 cm in 14 days at 23 C, radially furrowed, more or less wrinkled, consisting of a thick mycelial felt with floccose surface, in white to pale pink colors near Rosy Buff (Rayner, 1970) or Rosy Vinaceous (Rayner, 1970), developing a dense layer of cleistothecia on the felt, obscured by a loose overgrowth of aerial hyphae; conidia-bearing structures limited and not affecting the colony appearance; exduate lacking; odor slightly musty; reverse dull orange (Rosy Buff or Flesh; Rayner, 1970) with a deep red tint.

Colonies on malt extract agar growing rapidly, attaining a diameter of 4.2-4.6 cm in 14 days at 23 C, thin, more or less furrowed, floccose to velvety, with surface becoming granular by the development of abundant cleistothecia, in pale brown colors near Saffron (Rayner, 1970), with limited production of conidia; exduate lacking; odor indistinct; reverse pale yellowish brown near Honey or Isabelline (Rayner, 1970).

Colonies on oatmeal agar growing as on malt but thinner, granular by the development of abundant cleistothecia, with limited conidia, in pale yellow-orange colors near Salmon (Rayner, 1970); exduate abundant at submarginal areas, clear; odor lacking; reverse uncolored or somewhat dull reddish orange (Rust; Rayner, 1970).

Cleistothecia superficial, scattered or aggregated in small clusters, pale yellowish brown, subglobose to elongate, 150-280 µm in diam, glabrous or loosely covered with pigmented hyphae, maturing rather slowly from the center outwards, within 21 days. Peridium at first sclerotioid, consisting of masses of thick-walled, polygonal cells, later becoming thin and membranaceous. Asci typically borne in curved chains which consist of up to 6-7 cells, arising as branches from ascogenous hyphae, 8-spored, subglobose to ovate, 9-11.5 × 8-10 µm, evanescent at maturity. Ascospores hyaline to pale yellow, broadly lenticular, 3.0-4.0 × 2.5-3.0 µm, with equatorial areas flattened to show a shallow furrow but distinct crests lacking, with convex surfaces distinctly reticulate under SEM observation.

Penicilli (conidia-bearing structures) monoverticillate to mostly biverticillately asymmetrical. Conidiophores hyaline, smooth-walled, septate, variable, arising either from the substratum, 350-450(-900) × 2.5-3.0(-3.5) µm, or as short branches from aerial hyphae mostly less than 80 µm in length. Branches sometimes present, 25-35 µm in length. Metulae commonly in groups of 2-4, measuring 12.5-15(-20) × 2.5-4.0 µm, hyaline, smooth-walled. Phialides flask-shaped, 3-4 in the verticil, 9-12.5 × 2.0-2.5 µm. Conidia hyaline, globose to subglobose, 2.0-3.0 µm in diam, smooth-walled, connective in tangled or loosely column chains. Chlamydospores abundantly produced, mostly intercalary.

At 37 C, growth is nil.

Isolation: marine sludge, the Suez Canal, 30 km north from Port Said, Sinai Peninsula, Egypt, 9.x.1978, coll. H. Komatsu, No. 2894, NHL (holotype).

In his monographic studies, Scott (1968) pointed out that, on the basis of ascus arrangement within the cleisto-

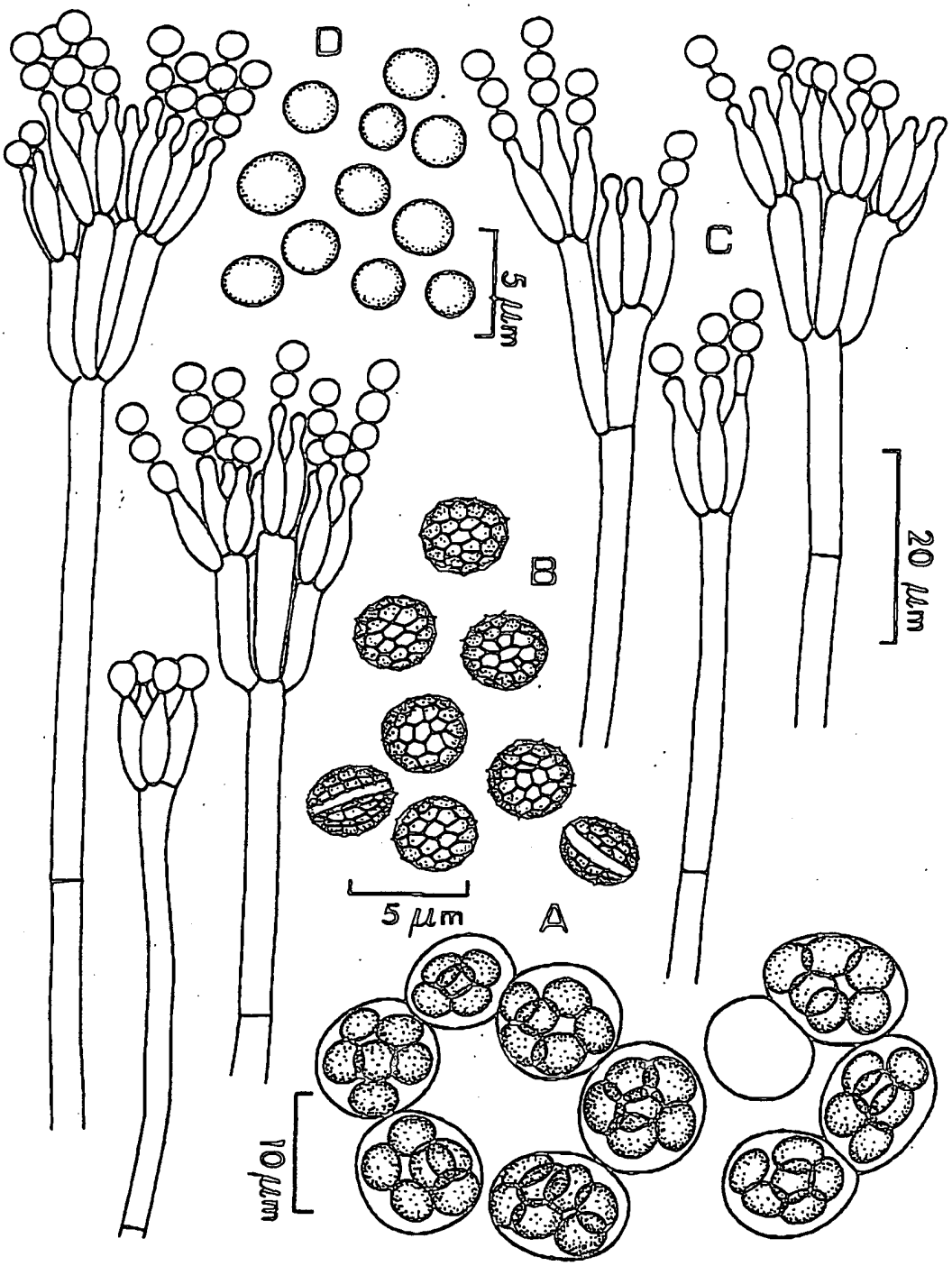
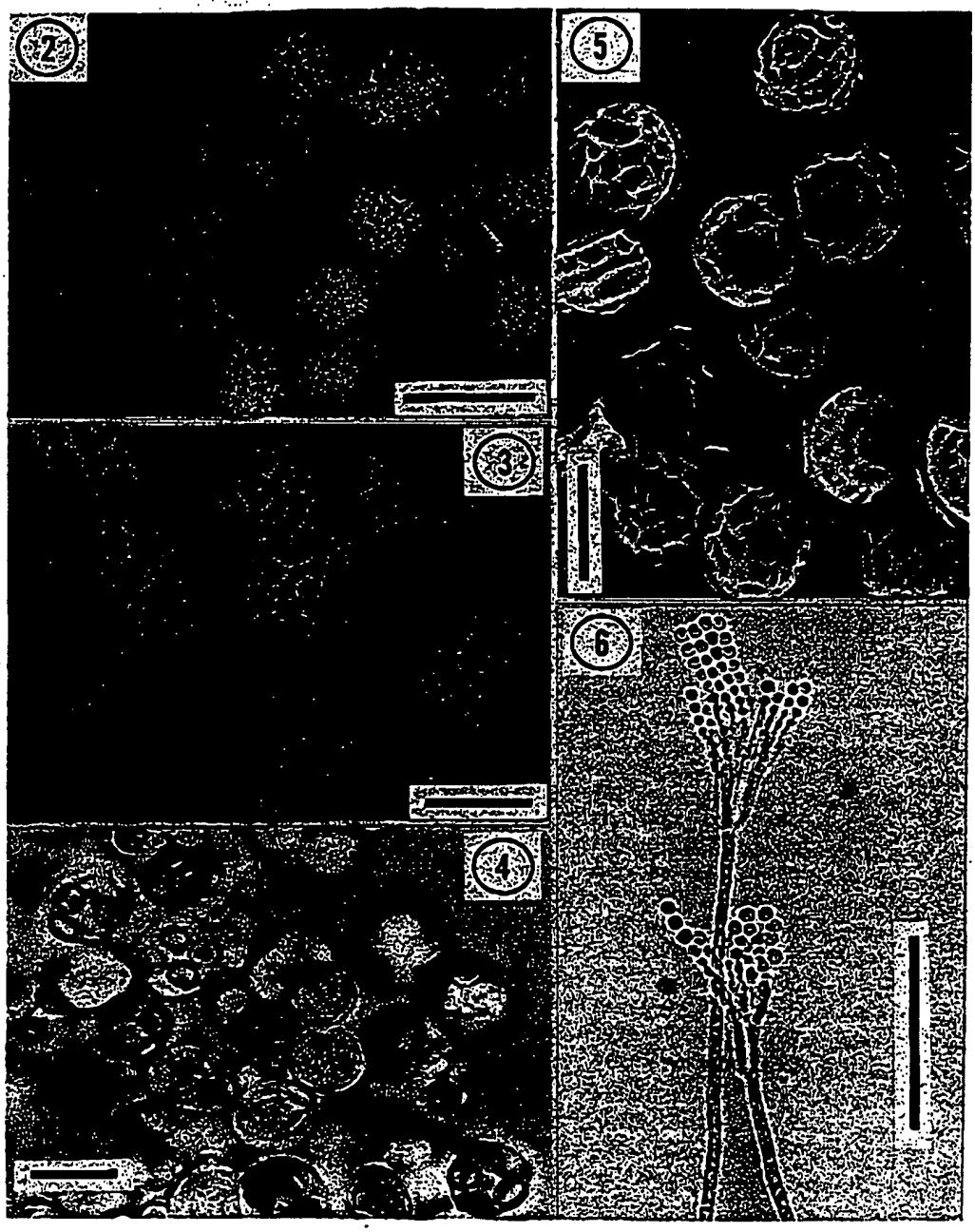



Fig. 1. *Eupenicillium sinaicum*, NHL 2894.
A. Asci. B. Ascospores. C. Penicilli. D. Conidia.

thecia, the species of *Eupenicillium* can be divided into two distinct series: one with species producing catenate asci, and other with asci borne singly. A scanning electron microscopic observation of the ascospore ornamentation for 21 *Eupenicillium* species was given by Udagawa and Horie (1973a), who proposed that the ascospore morphology should be the primary criterion for species separation. They separated the genus into two major groups of species, those bearing distinct equatorial crests and those without such ornamentation.

Pitt (1979), however, stated that 'such characters sometimes appear to have little relationship with other characters considered to be important in the classification of similar fungi, such as growth rates, colony morphology and anamorphic states'. We support the opinion that morphologies for all reproductive states should be consulted at the species level. Neverthless, in the taxonomy of *Eupenicillium*, the above features of asci and ascospores provide a useful basis for species separation. As an easily discernible and very stable feature, ascospore morphology traditionally has been used in the identification of species in other genera with cleistothecial ascocarps, particularly in the Eurotiaceae, e.g. *Eurotium*, *Neosartorya*, *Talaromyces*, etc. In our experience, growth rates of *Eupenicillium* species, even under standard conditions, are often unreliable. Conidia-bearing structures are sometimes lacking in fresh isolates of *Eupenicillium* species and are extremly inconspicuous or fragmentary in some strains of *E. ornatum* Udagawa, *E. meloforme* Udagawa & Horie, *E. parvum* (Raper & Fennell) Stolk & Scott, *E. rubidurum* Udagawa & Horie, etc. (Udagawa, 1968; Udagawa and Horie, 1973b).

*Eupenicillium sinaicum* becomes the fourth species of the genus known to produce ascospores with a reticulate wall. *Eupenicillium molle* Malloch & Cain (1972) has asci disposed in curved or helicoid chains, and appears to be the most closely related species. The ascospores of both of these species have nearly the same range in length and width. However, *E. molle* is clearly different from this species: the asci are smaller; the ascospores have 2 low equatorial ridges and a more irregular ornamentation (as can be seen by comparing Figs. 2, 3, 5, and Fig. 8 in the paper of Udagawa and Horie, 1973a); the penicilli vary in complexity from biverticillate, composed of terminal verticils of phialides numbering up to 8 or 10, to more variously branched and terverticillate, but less commonly have the monoverticillate pattern of *E. sinaicum*.




Figs. 2-4 and 6. *Eupenicillium sinaicum*, NHL 2894. 2, 3. SEM micrographs of ascospores. 4. Asci. 6. Penicilli. Fig. 5. SEM micrograph of ascospores of *Eupenicillium molle* Malloch & Cain, IMI 84589.

(scale-lines: 2=5 µm, 3=2 µm, 4=10 µm, 5=3 µm and 6=50 µm.)

The reticulate ascospore ornamentation of *E. sinaicum* is also somewhat similar to that of *E. meloforme* Udagawa & Horie (1973b) and *E. reticulisporum* Udagawa (1968) but differs in the lack of equatorial ridges. This species is primarily distinguished from the latter two species by the formation of its asci in helicoid chains.

## ACKNOWLEDGMENT

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## REFERENCES

Malloch, D., and R. F. Cain. 1972. New species and combinations of cleistothecial Ascomycetes. Can. J. Bot. 50: 61-72.

Pitt, J. I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London. 634 p.

Raper, K. B., and C. Thom. 1949. A manual of the Penicillia. Williams & Wilkins Co., Baltimore. 875 p.

Rayner, R. W. 1970. A mycological colour chart. Commonwealth Mycological Institute, Kew.

Scott, D. B. 1968. The genus *Eupenicillium* Ludwig. Microbiol. Res. Group, Bull. 1, Council for Scientific and Industrial Research, South Africa. Pretoria. 150 p.

Udagawa, S. 1968. Three new species of *Eupenicillium*. Trans. mycol. Soc. Japan 9: 49-56.

Udagawa, S., and Y. Horie. 1973a. Surface ornamentation of ascospores in *Eupenicillium* species. Antonie van Leeuwenhoek 39: 313-319.

Udagawa, S., and Y. Horie. 1973b. Some *Eupenicillium* from soil of New Guinea. Trans. mycol. Soc. Japan 14: 370-387.

Ueda, S. 1980. A mycofloral study on brackish water sediments in Nagasaki, Japan. Trans. mycol. Soc. Japan 21: 103-112. (Japanese)